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**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Application Number: 10/578,613
Filing Date: March 12, 2007
Appellant(s): TOHATA ET AL.

Stephen G. Baxter
For Appellant

**EXAMINER'S ANSWER**

This is in response to the appeal brief filed 12/15/2010 appealing from the Office action mailed 06/17/2010.

**(1) Real Party in Interest**

The examiner has no comment on the statement, or lack of statement, identifying by name the real party in interest in the brief.

**(2) Related Appeals and Interferences**

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

**(3) Status of Claims**

The following is a list of claims that are rejected and pending in the application: Claims 1-15.

**(4) Status of Amendments After Final**

The examiner has no comment on the appellant's statement of the status of amendments after final rejection contained in the brief.

**(5) Summary of Claimed Subject Matter**

The examiner has no comment on the summary of claimed subject matter contained in the brief.

**(6) Grounds of Rejection to be Reviewed on Appeal**

The examiner has no comment on the appellant's statement of the grounds of rejection to be reviewed on appeal. Every ground of rejection set forth in the Office action from which the appeal is taken (as modified by any advisory actions) is being maintained by the examiner except for the grounds of rejection (if any) listed under the subheading "WITHDRAWN REJECTIONS." New grounds of rejection (if any) are provided under the subheading "NEW GROUNDS OF REJECTION."

**(7) Claims Appendix**

The examiner has no comment on the copy of the appealed claims contained in the Appendix to the appellant's brief.

**(8) Evidence Relied Upon**

| | | |
|---|---|---|
| WO 03/083125 | Ferrari et al. | 10-2003 |

Gardan, R. et al. "Role of transcriptional activator *RocR* in the arginine-degradation pathway in *Bacillus subtilis*" Mol. Microbiol., 1997, vol. 24, pp. 825-837.

Hakamada et al. "Deduced amino acid sequence and possible catalytic residues of a thermostable, alkaline cellulase from an alkaliphilic *Bacillus* strain" Biosci. Biotechnol. Biochem., 2000, vol. 64, pp. 2281-2289.

**(9) Grounds of Rejection**

The following ground(s) of rejection are applicable to the appealed claims:

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ferrari et al. (WO 03/083125), in view of both Gardan et al. (Mol. Microbiol., 1997, 24: 825-837) and Hakamada et al. (Biosci. Biotechnol. Biochem., 2000, 64: 2281-2289).

Ferrari et al. teach a method of producing a secreted protein by using a recombinant *Bacillus subtilis*, wherein the recombinant *Bacillus subtilis* comprises a deletion of one or more genes, wherein the one or more genes could be *rocA, rocD* and/or *rocF*, and wherein the deletion of *rocA, rocD* and/or *rocF* results in enhanced levels of protein production over the wild type *Bacillus subtilis*. Ferrari et al. teach that the recombinant *Bacillus subtilis* further comprises a gene encoding a heterologous protein (i.e., the gene comprises a transcription initiation region), wherein the heterologous protein could be a cellulase (claims 1-3, 7-9, 12, and 13) (p. 1, line 33 to p.2, line 35; p. 3, lines 3-7; p. 36, lines 21-36). The gene encoding the heterologous protein further comprises a translation initiation region and a secretion signal region (claims 3 and 4) (p. 36, lines 3-7).

Ferrari et al. do not teach deleting *rocR* or *sigL* (claims 1, 10, 11, 14, and 15). However, doing such is suggested by the prior art. For example, Gardan et al. teach that *rocR* and *sigL* act in concert to activate transcription of the *rocA, rocD* and *rocF* genes (Abstract, p. 825, column 2, first full paragraph). Based on these teachings, one of skill in the art would have known that deleting either *rocR* or *sigL* would achieve the same effect as deleting *rocA, rocD* and *rocF*, i.e., eliminating the activity of the *rocA, rocD* and *rocF* genes. It would have been obvious to one of skill in the art, at the time the invention was made, to modify the *Bacillus subtilis* of Ferrari et al. by deleting *rocR* or *sigL* to achieve the predictable result of inactivating *rocA, rocD* and *rocF* and obtaining a microorganism suitable for protein production.

Ferrari et al. and Gardan et al. do not specifically teach using the cellulase transcription initiation, translation initiation and secretion signal regions as set forth by SEQ ID NO: 1 (claims 5 and 6). However, SEQ ID NO: 1 which encodes a cellulose was known in the prior art (see Hakamda et al., p. 2283, column 2, p. 2284, Fig. 1; see also the enclosed sequence alignment). It would have been obvious to one of skill in the art, at the time the invention was made, to modify the *Bacillus subtilis* of Ferrari et al. by using SEQ ID NO: 1 as the gene encoding the heterologous cellulase to achieve the predictable result of obtaining a microorganism suitable for the production of secreted cellulase. By doing such, one of skill in the art would have used the cellulase transcription initiation, translation initiation and secretion signal regions set forth by the nucleotides 1-659 of SEQ ID NO: 1.

Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

**(10) Response to Argument**

The arguments are answered below in the order in which they were presented in the Appeal Brief.

The appellant argues that it was far from predictable what the effects of knocking out *rocR* or *sigL* would have been and there was no reasonable expectation of success for obtaining a microbial strain that expresses at least 200% more heterologous protein than an unmodified strain.

This is not found persuasive because Ferrari et al. teach that knocking out *rocA*, *rocD* and/or *rocF* results in enhanced heterologous protein expression as compared to the unmodified strain. Gardan et al. indicate that knocking out *rocR* or *sigL* would also knock out *rocA, rocD* and *rocF*. Thus, one of skill in the art would have reasonably expected that knocking out *rocR* or *sigL* would be equivalent to knocking out *rocA, rocD* and *rocF* and that both would result in the same enhanced heterologous protein expression. Apart from an argument, the appellant did not provide any evidence to the contrary.

The appellant argues that there is no express teaching, suggestion or motivation in the prior art to knock out *sigL* or *rocR* to produce a *Bacillus* strain that expresses a higher amount of a heterologous protein than a strain not having *sigL* or *rocR* knock out.

Thus, the appellant argues, the rejection based on Ferrari, in view of Gardan fails the first prong of the test in *In re* Vaeck, 20 USPQ2d 1438 (Fed. Cir. 1991), specifically that the references themselves or the knowledge in the art must provide some suggestion or motivation to arrive at the invention.

This is not found persuasive because it is just an argument not supported by any evidence. Ferrari et al. teach eliminating the activity of *rocA, rocD* and/or *rocF* by knocking out these gene from the bacterial chromosome, wherein knocking out *rocA, rocD* and/or *rocF* results in enhanced heterologous protein expression. Gardan et al. teach that *rocR* and *sigL* act in concert to activate the transcription of the *rocA, rocD* or *rocF* genes. Because *rocA, rocD* or *rocF* require both *sigL* and *rocR* for their expression, one of skill in the art would have known that deleting either *rocR* or *sigL* would eliminate the activity of the *rocA, rocD* and *rocF* genes. Since Ferrari et al. teach that eliminating the activity of *rocA, rocD* and *rocF* results in enhanced expression of heterologous proteins, one of skill in the art would have known that knocking out either *rocR* or *sigL* (which would necessarily eliminate the activity of *rocA, rocD* and *rocF*) would also result in enhanced expression of heterologous proteins over a cell not having *sigL* or *rocR* knock out. Thus, suggestion to modify the teachings of Ferrari et al. is found in the cited references.

The appellant argues that the examiner has not articulated any reason why one of ordinary skill in the art would have selectively inactivated *rocR* or *sigL* (as a means of

selectively inactivating *rocA*, *rocF* or *rocD* of Ferrari) as opposed to the less complex route of directly knocking out the *rocA*, *rocF* or *rocD*.

In response, it is noted that knocking one of *rocR* and *sigL* is not more complex than knocking out one of *rocA*, *rocF* and *rocD*, as both involve approaches involve knocking out only one gene. Furthermore, Ferrari et al. teach deleting more than one gene. Since the prior art teaches that *rocR* and *sigL* act together to activate transcription from *rocA, rocD* or *rocF*, one of skill in the art would have known that knocking out either *rocR* or *sigL* would eliminate all three *rocA, rocD* and *rocF*. Thus, one of skill in the art seeking to eliminate the activity of more than one gene, as taught by Ferrari et al., would have chosen the less complex and more direct approach process of knocking out one gene (i.e., either *rocR* or *sigL*) than the more complex process of eliminating three genes (i.e., *rocA*, *rocF* and *rocD*). Apart from an argument, the appellant did not provide any evidence to the contrary.

The appellant argues that the examiner has engaged in hindsight reasoning using the present disclosure as a guide for specifically selecting an indirect knockout of a *rocA*, *rocF* or *rocD* gene regulated by *sigL* or *rocR*.

In response to appellant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does

not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). The instant rejection is not based on picking and choosing among isolated disclosures in the prior art. Specifically, Ferrari et al. teach knocking out *rocA*, *rocF* and/or *rocD* and Gardan et al. teach that knocking out all of *rocA*, *rocF* and *rocD* can be achieved by knocking out either *sigL* or *rocR*. At the time the invention was made, one of skill in the art being presented only with Ferrari et al. and Gardan et al., would have known that knocking out either *sigL* or *rocR* would achieve the same result as knocking out *rocA*, *rocF* and *rocD*. The accepted wisdom in the prior art would have guided one of skill in the art to modify Ferrari et al. by using the simpler approach of knocking out one gene (i.e., either *sigL* or *rocR*) as opposed to the more complex approach of knocking out all of *rocA*, *rocF* and *rocD*. Apart from an argument, the appellant did not provide any evidence to the contrary.

The appellant argues that the examiner has not established that, based on the cited prior art and the level of ordinary skill in the art that, one would have had a reasonable expectation of obtaining a *Bacillus* strain having an enhanced ability to express a heterologous protein by knocking out *sigL* or *rocR*. For example, assuming *arguendo* that knocking out *sigL* or *rocR* would have inactivated the operons containing the *rocA*, *rocF* and *rocD* genes of Ferrari et al., as presumed by the examiner, the examiner has not explained why this would have been expected to enhance protein synthesis by a *Bacillus* as opposed to either having no effect on protein synthesis or

crippling protein synthesis by the cell. The examiner has failed to point out any teaching in the prior art suggesting that knocking out one of these genes would have been expected to increase protein synthesis by inactivating arginine catabolism pathways.

This is not found persuasive. First, that knocking out *sigL* or *rocR* would inactivate the operons containing the *rocA*, *rocF* and *rocD* genes of Ferrari et al. is not a presumption but a certainty. This is because both *sigL* and *rocR* are necessary to activate *rocA*, *rocF* and *rocD* transcription. Second, since Ferrari et al. teach that eliminating the activity of *rocA, rocD* and/or *rocF* results in enhanced expression of heterologous proteins, one of skill in the art would have known that knocking out either *rocR* or *sigL* (which would necessarily eliminate the activity of *rocA, rocD* and *rocF*) would also result in enhanced expression of heterologous proteins. Apart from an argument, the appellant did not provide any evidence to the contrary.

The appellant argues that, despite the lack of any express teaching in the prior art to knock out either of *rocR* and *sigL*, the examiner asserts that doing such is suggested by the prior art. The appellant argues that this chain of hindsight reasoning is not disclosed or suggested by the prior art and there is significant unpredictability inherent to each step. According to the appellant, the examiner's chain of reasoning is outlined below:

**(1)** Deletion of *rocR* or *sigL* activators of *rocABC* and *rocDEF* operons.

The appellant argues that, although the examiner supposes that by knocking out expression of *rocR* or *sigL* the activation of the *rocABC* and *rocDEF* operons would

have been inhibited, the examiner has not established that microbes do not express other activators of these operons or explained why knocking out only one of these protein activators would have been expected to substantially inactivate expression of these operons. One of ordinary skill in the art would have expected that all activators of these operons would have to be knocked out to prevent the expression of proteins they express affecting arginine catabolism.

This is just an argument not supported by any evidence. It was common knowledge in the prior art that all that is needed to inactivate transcription of a gene is to inactivate an essential transcriptional activator of the gene. Gardan et al. teach that *rocR* and *sigL* act in concert as essential transcriptional activators of *rocABC* and *rocDEF* operons (see Abstract, p. 825, column 1, first full paragraph). Based on these teachings in the prior art, one of skill in the art would have known that knocking out either *rocR* or *sigL* would inactivate both the *rocABC* and *rocDEF* operons. Apart from an argument, the appellant did not provide any evidence to the contrary.

**(2)** Decreased activation of the *rocABC* or *rocDEF* operons thus inactivating the *rocA*, *rocD* and *rocF* genes and reducing the conversion or catabolism of arginine into glutamate.

The appellant argues that, assuming that knocking out *rocR* or *sigL* would have reduced the expression of the *rocABC* or *rocDEF* operons, the examiner has not established that this reduced expression would have had any effect on heterologous protein synthesis.

This argument is not found persuasive for the reasons set forth above. Specifically, Ferrari et al. teach that eliminating the activity of *rocA, rocD* and/or *rocF* results in enhanced expression of heterologous proteins. Since knocking out either *rocR* or *sigL* necessarily eliminates the activity of *rocA, rocD* and *rocF*, one of skill in the art would have known that knocking out either *rocR* or *sigL* would also result in enhanced expression of heterologous proteins. Apart from an argument, the appellant did not provide any evidence to the contrary.

**(3)** Less arginine catabolism by the cell.

The appellant argues that the examiner presumes that reduced expression of *rocABC* or *rocDEF* operons would reduce the amount of arginine catabolism by preventing its conversion into glutamate. However, the examiner has not established that the microbial cell lacked other redundant proteins for catabolizing arginine or that converting arginine into glutamate would reduce heterologous protein synthesis.

This is just an argument not supported by any evidence. The appellant just assumes that microbial cell have redundant proteins for catabolizing arginine or that converting arginine into glutamate would have reduced heterologous protein synthesis without any evidence to this effect. An obviousness-type rejection is based on the knowledge available in the prior art as a whole. The prior art of record indicates that knocking out *rocF* alone would be sufficient to inhibit conversion of arginine into glutamate; this is because *rocF* acts upstream, at the first step of the conversion pathway (see Fig. 1 in Belitski et al., Proc Natl Acad Sci USA, 1999, 96: 10290-10295, provided by the appellant with the reply filed on 04/07/2010). Furthermore, Fig. 1 in

Belitski et al. shows that *rocA, C, and D* act at different downstream steps in the conversion pathway. Since Gardan et al. teach that *sigL* and *rocR* are required to activate transcription from the *rocABC* or *rocDEF* operons, one of skill in the art would have certainly known that knocking out either *sigL* or *rocR*, as proposed by the instant rejection, would necessarily inhibit *rocA*, *C*, *D*, and *F* and thus, the conversion of arginine to glutamate.

The argument that the examiner did not establish that converting arginine into glutamate would reduce heterologous protein synthesis is not found persuasive. Belitski et al. teach that *rocA, C, D,* and *F* act at different steps in the arginine to glutamate conversion pathway and Ferrari et al. teach that knocking out *rocA, rocD* and *rocF* results in enhanced production of heterologous proteins. These combined teachings demonstrate that inhibiting the conversion of arginine to glutamate by knocking out *rocA, rocD* and *rocF* results in enhanced production of heterologous proteins. Thus, the teachings in the prior art of record establish that leaving the conversion of arginine to glutamate intact would reduce the production of heterologous protein.

**(4)** Accumulation of intracellular arginine available for protein synthesis.

The examiner presumes that knocking out *rocR* or *sigL* would have resulted in accumulation of arginine inside the cell. However, no support is established for this presumption. The appellant submits that he has shown that less arginine would accumulate into cells if RocC or RocE (which are involved in the import of arginine into the cell) were knocked out by inhibition of *rocABC* or *rocDEF*. The appellant argues

that, although the examiner addresses the argument above by stating that RocC and RocE are only necessary when arginine or certain other amino acids are the sole nitrogen sources, the examiner ignores that these proteins were known to be involved in the importation of arginine into a cell and that one of ordinary skill in the art would have expected that inhibiting their expression would have reduced the level of arginine inside the cell. The examiner has also failed to establish that were arginine catabolism or conversion to glutamate was inhibited, arginine would have accumulated in a pool available for protein synthesis instead of shunted into and removed into a different catabolic pathway.

Basically the appellant argues that arginine import into the cells is required for protein synthesis and that one of skill in the art would have expected that protein synthesis would be reduced in *Bacillus subtilis* cells with impaired RocC and RocE activity. However, it is the metabolism of arginine synthesized within the cells and not the arginine import into cells which affects protein synthesis. While it is true that cells lacking RocC and RocE activity cannot import arginine from the media into the cells, the specific import of arginine into the cells is not necessary for the intracellular synthesis of amino acid and proteins. It was common knowledge in the prior art that amino acids are synthesized inside the cells as long as a nitrogen source is provided into the media. Arginine is only used in media to provide the nitrogen necessary for the intracellular biosynthesis of nitrogen-containing molecules such as all amino acids necessary for protein synthesis. However, cells can synthesize these amino acids from various nitrogen sources beside arginine. This is evidenced by the prior art which teaches that

*Bacillus subtilis* having knocked out *sigL* (i.e., also lacking RocC and RocE activity and thus not being able to import arginine into cells) are viable and grow equally well on nitrogen sources other than arginine (Debarbouille et al., Proc Natl Acad Sci USA, 1991, 88: 9092-9096, provided by the appellant with the reply filed on 04/07/2010; see p. 9095, column 1, 6th full paragraph and paragraph bridging columns 1 and 2). Since cell growth is not affected this means that knocking out *sigL* does not adversely affect protein synthesis. Since protein synthesis is not adversely affected, arginine is synthesized inside the cells in the absence of RocC and RocE activity and the absence of arginine import into the cells. Furthermore, the prior art indicates that knocking out *sigL* would also knock out *rocA*, *rocD* and *rocF* (see the teachings of Gardan et al. above, evidencing that knocking out *sigL* inactivates both *rocABC* or *rocDEF* operons) and that *rocA*, *rocD* and *rocF* contribute to arginine conversion to glutamate within the cells (Gardan et al., see Fig. 1A; Belitski et al., Proc Natl Acad Sci USA, 1999, 96: 10290-10295, provided by the appellant with the reply filed on 04/07/2010, see Fig. 1). Thus, by knocking out *sigL*, one of skill in the art would have necessarily reduced the conversion of intracellularly-synthesized arginine into glutamate and cause arginine accumulation within the cells for enhanced protein synthesis. Based on the teachings in the art as a whole, one of skill in the art would have reasonably expected that inhibiting the expression of *sigL* or *rocR* would have increased the level of arginine inside the cell. Apart from an argument, the appellant did not provide any evidence to the contrary.

Although the appellant states that he has shown that less arginine would accumulate if RocC or RocE were to be inactivated, this statement is based on the

teachings of Debarbouille et al., Gardan et al., and Belitski et al. (see p. 5 of the final OA). As set forth above, the teachings of Debarbouille et al., Gardan et al., and Belitski et al. all indicate the contrary, i.e., that arginine accumulates into the cells when either *sigL* or *rocR* is knocked out.

**(5)** More heterologous protein synthesis.

The appellant argues that the examiner has presumed that an increase in intracellular arginine would have increased heterologous protein synthesis, but has not established that intracellular arginine levels in unmodified microbial cells were rate limiting for protein synthesis. If unmodified cells already had a surplus of arginine then increasing its accumulation in the cell would not have been expected to affect the amount of protein synthesis.

In response, the appellant just assumes that unmodified cells already have a surplus of arginine without providing any evidence to this effect. Importantly, as set forth above, the teachings in the prior art of record indicate that increasing the intracellular arginine pool would have increased the production of heterologous proteins.

The appellant argues that one of skill in the art would not have expected to increase protein synthesis by knocking out *sigL* or *rocR* because less arginine would have been expected to be transported into the cell. Less arginine transport would have been expected to result in a lower intracellular level of arginine being available for protein synthesis and thus lower protein synthesis.

This argument has been addressed above and it is not found persuasive for the reasons set forth above.

The appellant argues that, while the examiner states at the bottom of page 6 of the final OA that *Bacillus* cells need not use arginine as a nitrogen source, this argument confuses the ability of a cell to survive (grow) on a medium containing a particular nitrogen source with the ability of a cell to express higher amounts of a heterologous protein. It does not follow that a cell that can grow on an arginine-free medium (e.g., a cell where the arginine catabolism pathways are knocked out to boost the pool of arginine) will produce a higher amount of a heterologous protein than a cell that contains these active pathways. While it may be perfectly reasonable to expect that a *Bacillus* that does not express arginine permease (or *roc* genes that catabolize arginine) will grow on a medium containing an alternative nitrogen source, the examiner has not pointed out any teaching in the prior art that indicates that heterologous protein synthesis would be enhanced in such cells. For example, one of ordinary skill in the art would have recognized that cells not able to take in arginine from the medium would have to expend metabolic resources to synthesize arginine they incorporate into a heterologous protein. Cells that can obtain arginine freely from the medium would not be subject to the same metabolic costs and would be expected to more efficiently express heterologous proteins.

These arguments are not found persuasive. Not only are these arguments unsupported by any evidence, but the prior art indicates the contrary. Specifically,

Belitski et al. teach that *rocA, D,* and *F* act at different steps in the pathway of arginine conversion to glutamate. Ferrari et al. teach that knocking out *rocA, D* and *F* (i.e., inhibiting arginine conversion to glutamate) results in enhanced production of heterologous proteins over cells with intact *rocA, D* and *F*. Thus, the prior art of record does teach that inhibiting the catabolism of arginine to glutamate results in enhanced production of heterologous proteins and that leaving the arginine to glutamate conversion pathway intact would have reduced the production of heterologous protein. The prior art of record teaches that cells capable of growing in an arginine-free medium (e.g., where the arginine catabolism pathway are knocked out) do produce a higher amount of a heterologous protein than cells containing the active arginine catabolism pathway. Based on the teachings, one of skill in the art would have known that it is the catabolism of intracellularly- synthesized arginine and not the arginine import into cells which affects protein synthesis. For the same reasons, the argument that cells that can obtain arginine freely from the medium would be expected to more efficiently express heterologous proteins is not found persuasive. The prior art demonstrate the contrary.

The appellant argues that, in the Examples in the present application, nitrogen sources contained in the culture medium used were tryptone and yeast extract, both of which are generally used and well-balanced nitrogen sources. This culture medium is not a specific medium and if some amino acids such as arginine, isoleucine, or valine are exhausted, the medium does not provide substitutive amino acids. Although other amino acids in the medium may substitute for the exhausted amino acids as alleged by

the examiner, this would only be possible during the early phase of culturing when such amino acids are present (i.e., not yet depleted). In the Examples, the protein productivity of host cells was investigated after 5 day culturing at which time the available nitrogen source in the culture medium had been depleted. Under the culture conditions described above, i.e., the condition in which cells were cultured long enough to deplete all available nitrogen sources in a medium having a normal amino acid content, one would have expected that the protein productivity of the knocked-out cells which cannot use arginine, isoleucine, or valine would have been lower that that of normal cells which can use these amino acids because these knock-out mutations would limit the access to nitrogen contained in these amino acids. Therefore, even taking into account the examiner's allegation, the higher protein productivity of the claimed microorganism is surprising.

These arguments are not supported by any evidence and are thus not persuasive. Specifically, there is no evidence in the instant specification that the cells were cultured long enough such as to deplete all available nitrogen sources or, in alternative, that the cells can survive for several days in a medium depleted of all nitrogen sources, which nitrogen sources are essential for viability, growth and protein synthesis within the cells. The Examples provided by the instant specification only disclose that 0.03 ml of cell suspensions were inoculated into 2 L of nutrient-containing medium (i.e., inoculated into an excess of nutrient-containing medium of approximately 67,000,000 x) and that the cells were cultured for 3-6 days. However, the instant specification does not indicate that, at the time of the harvest (i.e., 3-6 days), the cells

grew at a density such as to deplete all nitrogen sources from this enormous excess of nutrient-containing medium. Furthermore, the appellant did not provide any evidence that using arginine, isoleucine, or valine is absolutely required for enhanced protein production. In fact, Ferrari et al. provide evidence to the contrary, as they teach that cells not able to use arginine as a source of nitrogen (i.e., cells wherein the arginine catabolic pathway is inhibited) are capable of enhanced production of heterologous proteins, as these cells use nitrogen sources other than arginine to synthesize and accumulate amino acids, including arginine, within the cells. Consequently, arginine permease activity (i.e., arginine import into the cells) is not necessary for enhanced protein production, as the cells of Ferrari et al. do not rely on extracellular arginine for enhanced protein production. One of skill in the art would have reasonably expected that knocking out *rocR* or *sigL* would have resulted in higher cytoplasmic levels of arginine and higher levels of protein expression because knocking out *rocR* or *sigL* would also inactivate arginine catabolic pathway and thus more intracellularly-produced arginine would be available for protein synthesis.

The appellant argues that he has shown that deletion of the *sigL* or *rocR* produce microorganisms which express surprisingly higher levels of the heterologous protein than the corresponding wild-type microorganism not having these deletions. The superior productivity of the microorganism according to the invention is shown in Table 4 of the specification. The appellant argues that the prior art provides no

expectation of success for the significantly higher levels of heterologous proteins produced by the recombinant microorganisms of the invention.

This is not found persuasive. The instant rejection is based on Ferrari et al. who teach cells with knocked out *rocA, rocD* and/or *rocF* and on modifying Ferrari et al. by knocking out *rocR* or *sigL* instead of knocking out *rocA, rocD* and/or *rocF*. Thus, for the argument of surprising results to be persuasive, the appellant must provide evidence that knocking out *rocR* or *sigL* provides superior protein production over knocking out *rocA, rocD* and/or *rocF* as taught by Ferrari et al. and not over wild type cells not having knocked out *rocR* or *sigL*. Importantly, that deletion of either *sigL* or *rocR* would produce microorganisms expressing levels of heterologous proteins that are higher than the levels in the corresponding wild-type microorganism not having these deletions was to be expected from the teachings in the prior art. Specifically, similar to the instant specification, Ferrari et al. teach that knocking out *rocA, rocD* or *rocF* results in levels of heterologous proteins that are higher than the levels in the corresponding wild-type microorganism not having these deletions. Gardan et al. indicate that knocking out *rocR* or *sigL* would also knock out *rocA, rocD* and *rocF*. Thus, it would have been expected that inactivating *rocA, rocD* and *rocF* by knocking out *rocR* or *sigL* would result in the same enhanced heterologous protein expression over the wild type cells, as taught by Ferrari et al. The appellant did not provide any evidence to the contrary.

The appellant argues that Hakemada et al. do not remedy the deficiencies in the two primary references. This is not found persuasive because, as set forth above, there is no deficiency to be remedied.

**In conclusion**, the instant rejection is clearly based on the knowledge within the level of one of skill in the art at the time the invention was made. The claims under appeal are drawn to a method of producing a protein by using compositions and steps well described in the prior art. Ferrari et al. teach a method of producing a heterologous protein by using a recombinant *Bacillus subtilis* comprising a gene encoding the heterologous protein and inactivated *rocA, rocD* and/or *rocF* genes, wherein the inactivation of *rocA, rocD* and/or *rocF* genes results in enhanced levels of heterologous protein production over the wild type *Bacillus subtilis*. While Ferrari et al. do not teach inactivating *sigL* or *rocR*, Gardan et al. teach that inactivating either of these two genes results in the inactivation of *rocA, rocD* and *rocF* genes. One of skill in the art would found it obvious to use routine experimentation to inactivate either *sigL* or *rocR* to achieve the predictable result of inactivating *rocA, rocD* and *rocF* and obtaining a microorganism suitable for enhanced protein production.

Please note that, as set forth above, neither the art as a whole nor the instant specification supports the appellant's arguments of unpredictability, lack of reasonable expectation of success, and superior results.

Thus, the Office has properly applied the test of obviousness set forth in *Graham v. John Deere Company* and established a *prima facie* case of obviousness over the

claims on appeal based on the teachings of Ferrari et al. taken with both Gardan et al. and Hakamada et al.

**(11) Related Proceeding(s) Appendix**

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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